

1. A process for the preparation of an aqueous liquid comprising a phytase, the process comprising:
- 5 (a) culturing in an aqueous medium a microorganism of the genus *Aspergillus* or *Trichoderma* having a heterologous phytase gene under the control of a glucoamylase (for *Aspergillus*) or cellobiohydrolase (for *Trichoderma*) promoter, under conditions that allow recombinant expression of the phytase, where the medium comprises, as a feed for the microorganism an assimilable carbon source and an
- 10 assimilable nitrogen source;
- (b) filtering the aqueous medium to remove the microorganisms to give an aqueous filtrate; and
- (c) subjecting the filtrate from (b) to ultrafiltration to give an aqueous liquid, having a phytase concentration of at least about 14,000 FTU/g.
- 15 2. A process according to claim 1 where the microorganism is *Aspergillus niger*, *Aspergillus oryzae* or *Trichoderma reesei*.
3. A process according to claim 1 wherein the microorganism does not possess, or does not express, a glucoamylase (AG) gene.
4. A process according to claim 1 wherein the microorganism possesses
- 20 multiple copies of the phytase gene.
5. A process according to claim 1 wherein the aqueous liquid is substantially free of taka-amylase.
6. A process according to claim 1 wherein substantially all of the carbon and nitrogen sources in the medium have been consumed by the microorganisms
- 25 before filtering in (b).
7. A process according to claim 1 wherein the aqueous liquid is free of the carbon and/or nitrogen sources:
8. A process according to claim 1 wherein the phytase is expressed in the microorganism with a glucoamylase signal sequence.
- 30 9. A process according to claim 1 wherein neither the aqueous filtrate or the aqueous liquid are subjected to: (a) crystallisation; (b) a colour-removal step; or (c) crystallization and a colour removal step.

10. A process according to claim 1 wherein the resulting aqueous liquid has a phytase activity of about 18,000 FTU/g or more.

11. An aqueous liquid prepared by a process according to claim 1 comprising a phytase at a concentration of at least about 14,000 FTU/g.

5 12. An aqueous liquid according to claim 11 which is derived from a culture medium in which the phytase was expressed.

13. A process for the preparation of a phytase-containing granulate, suitable for use in an animal feed, the process comprising processing a solid carrier comprising at least about 15% (w/w) of an edible carbohydrate polymer and an
10 aqueous liquid according to claim 11 to obtain phytase-containing granules.

14. A process according to claim 13 that comprises mixing the aqueous liquid and carrier, and kneading the resulting mixture.

15. A process according to claim 14 wherein the granules are subsequently dried.

15 16. A process according to claim 13 wherein the process comprises:

- (a) mixing the aqueous liquid containing the phytase with the solid carrier;
- (b) mechanically processing the mixture obtained in (a) to obtain enzyme-containing granules; and
- (c) drying the enzyme-containing granules obtained in (b).

20 17. A process according to claim 15 wherein the processing comprises a method selected from the group consisting of extrusion, pelleting, high-shear granulation, expansion, and fluid bed agglomeration.

Suhai 18. A phytase-containing granulate prepared by a process according to claim 13.

Suhai 25 19. A granulate comprising dried granules formed from a phytase and a solid carrier which comprises at least about 15% (w/w) of an edible carbohydrate polymer.

20. A granulate according to claim 19 wherein the granules comprise at least one divalent cation.

30 21. A granulate according to claim 19 wherein the granules comprise one or more hydrophobic, gel-forming or water insoluble compound(s).

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23. A granulate according to claim 22 wherein: (a) the derivatised cellulose is selected from the group consisting of hydroxy-propyl-methyl-cellulose, carboxy-methyl-cellulose and hydroxy-ethyl-cellulose; (b) the edible oil is soy oil or canola oil; or (c) the derivatised cellulose is as according to (a) and the edible oil is as according to (b).

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starch.

heat tolerant (thermostable) phytase.

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derived from an *Aspergillus* or *Trichoderma* species.

20

optionally is dried.

25

Sub
a2 ETU

~~ETU/g; or~~

30

composition.

33. A composition according to claim 31 which is an animal feed.

Sub 13

34. A composition according to claim 31, wherein said composition comprises pellets that comprise one or more feed substance(s) or ingredient(s) mixed with a granulate that comprises dried granules formed from a phytase and a solid carrier which comprises at least about 15% (w/w) of an edible carbohydrate polymer.

5 35. A composition according to claim 31 which is an animal feed, or a premix or precursor to an animal feed, and is prepared by a process that comprises mixing a phytase-containing granulate with one or more animal feed substance(s) or ingredient(s).

36. A process for promoting the growth of an animal, wherein said process
10 comprises feeding an animal with a diet that comprises:

(a) a granulate according to claim 19;

(b) a phytase-containing granulate with an activity of at least about 6,000 FTU/g;

15 or (c) both a granulate according to (a) and a phytase-containing granulate according to (b).

37. A process for promoting the growth of an animal, wherein said process comprises feeding said animal with a granulate as defined in claim 19 in, or as a component of, an animal feed, or in an animal diet.

20 38. A method for improving the pelleting stability of phytase, wherein said method comprises formulating said phytase in a composition comprising at least about 15% (w/w) of an edible carbohydrate polymer as a carrier for said phytase.

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